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(54) Title: <b>DETECTION OF THE LEPTIN RECEPTOR IN REPRODUCTIVE ORGANS AND METHODS FOR REGULATING REPRODUCTIVE BIOLOGY</b>			
(57) Abstract			
<p>The present invention relates to variant forms of the receptor for the <i>obese</i> gene product. In particular, the invention relates to methods of detecting receptor variants in the reproductive organs for the diagnosis of the cause of infertility. In addition, it relates to methods of inhibiting or down-regulating expression of defective variants in cells to augment their responsiveness to regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system to improve fertility.</p>			

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DETECTION OF THE LEPTIN RECEPTOR  
IN REPRODUCTIVE ORGANS AND METHODS  
FOR REGULATING REPRODUCTIVE BIOLOGY

1. INTRODUCTION

5 The present invention relates to variant forms of the receptor for the *obese* gene product. In particular, the invention relates to methods of detecting receptor variants in the reproductive organs for the diagnosis of the cause of infertility. In addition, it relates to methods of 10 inhibiting or down-regulating expression of defective variants in cells to augment their responsiveness to regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated 15 with this ligand-receptor system to improve fertility.

2. BACKGROUND OF THE INVENTION

Infertility is a major clinical problem in Western societies. A number of contributing factors have been identified for infertility, which include metabolic diseases 20 in a male that result in insufficient sperm production and the inability of a female's ovaries to produce or release ova. In addition, pituitary disorders may cause infertility in both sexes because the gonads are responsive to regulation by pituitary hormones such as follicle-stimulating hormone 25 and luteinizing hormone. However, clinical conditions exist in which an infertile female is still capable of ovulating. Therefore, the ovary may respond to additional signals that are not yet identified.

30 Zhang et al. (1994, *Nature* 372:425-432) describe the cloning and sequencing of the mouse *ob* gene and its human homolog. In an effort to understand the physiologic function of the *ob* gene, several independent research groups produced recombinant *ob* gene product in bacteria for *in vivo* testing 35 (Pelleymounter et al., 1995, *Science* 269:540-543; Halaas et al., 1995, *Science* 269:543-546; Campfield et al., 1995, *Science* 269:546-549). When the *Ob* protein (also known as leptin) was injected into grossly obese mice, which possessed

two mutant copies of the *ob* gene, the mice exhibited a reduced appetite and began to lose weight. Similarly, when normal mice received leptin, they also ate less than the untreated controls. Interestingly, when leptin was 5 administered to *ob/ob* female mice which were always infertile, fertility was restored in these animals (Chehab et al., 1996, *Nature Genetics* 12:318-320).

Recently, a leptin fusion protein was generated and used to screen for the leptin receptor (also known as OB-R) in a 10 cDNA expression library prepared from mouse choroid plexus, a tissue that lines brain cavities termed ventricles (Tartaglia, 1995, *Cell* 83:1263-1271). This approach led to the cloning of one form of the OB-R coding sequence, which reveals a single membrane-spanning receptor, sharing 15 structural similarities with several Class I cytokine receptors, such as the gp130 signal-transducing component of the interleukin-6 receptor (Taga et al., 1989, *Cell* 58:573-581), the granulocyte-colony stimulating factor receptor (Fukunaga et al., 1990, *Cell* 61:341-350), and the leukemia 20 inhibitory factor receptor (Gearing et al., 1991, *EMBO J.* 10:2839-2848). Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) demonstrate that OB-R mRNA is expressed in several tissues, including lung, kidney, total brain, choroid plexus and hypothalamus.

25 The reported mouse OB-R protein contains a relatively short intracellular cytoplasmic domain as compared with other Class I cytokine receptors. Subsequently, when cDNA encoding its human homolog was isolated from a human infant brain library, the predicted human protein sequence contains a much 30 longer intracellular domain. In view of this finding, it was speculated that different forms of the receptor might exist (Barinaga, 1996, *Science* 271:29). However, prior to the present invention, there was no report on how variant forms of the OB-R in humans would relate to infertility.

3. SUMMARY OF THE INVENTION

The present invention relates to variant forms of the human OB-R. In particular, it relates to the detection of these receptor variants in reproductive organs such as the 5 ovary and the prostate gland for diagnosis of the cause of infertility, and methods for treating infertility by targeting these variant receptors.

The invention is based, in part, upon the Applicants' discovery of human cDNA clones encoding three variant forms 10 of the OB-R. These receptors differ structurally from a reported OB-R with only three amino acid substitutions in the extracellular domain, but extensive diversity is observed in their intracellular cytoplasmic domains at the 3' end. The cytoplasmic domains of the variants of the invention are both 15 shorter and distinct in nucleotide sequence from the corresponding domain of the published form of OB-R (Tartaglia et al., 1995 Cell 83:1263). In addition, the cytoplasmic domain of one such variant is highly homologous to a human retrotransposon sequence. The OB-R variants described herein 20 represent incomplete receptors which may be incompetent or partially competent in transducing signals upon ligand binding. Expression of the different forms of the receptor have been detected in prostate and ovary. Furthermore, 25 leptin activity has been shown to be naturally present in ovarian follicular fluids. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the detection of the receptor variants in reproductive organs for the diagnosis of infertility, methods to inhibit and/or down-regulate the expression of these 30 receptor variants, gene therapy to replace the receptor variants in homozygous individuals, and direct activation of downstream signal transduction pathways in cells expressing the defective receptor variants for improving fertility.

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4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1E. Nucleotide sequence and deduced amino acid sequence of Form 1 the human OB-R variant.

5 The amino acid sequence diverges from the human OB-R reported by Tartaglia et al. (1995, Cell 83:1263-1271) at nucleotide residue #349, #422, #764 and from residue #2770 and beyond.

5

Figure 2. Nucleotide sequence comparison between human OB-R variant Form 1, Form 2 and Form 3 at the 3' end.

10 Figure 3A-3C. Amino acid sequence comparison between OB-R variant Forms 1 (HuBl.219-1), 2 (HuBl.219-2), 3 (HuBl.219-3), human OB-R (HuOBR) published by Tartaglia et al., 1995, Cell 83:1263; and murine OB-R (MuOBR).

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Figure 4. Proliferation of BaF3 cells and cells transfected with chimeric OB-R in the presence of follicular fluids. ■ = transfected cell line; □ = BaF3 parent cell line.

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Figure 5. Proliferation of transfected BaF3 cell line in the presence of follicular fluids is inhibited by soluble OB-R, indicating that leptin is the active growth-inducing substance in the fluids. ■ = transfected cell line; □ = BaF3 parent cell line.

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5. DETAILED DESCRIPTION OF THE INVENTION  
5.1. THE OB-R VARIANTS

30 The present invention relates to nucleic acid and amino acid sequences of OB-R variants. In a specific embodiment by way of example in Section 6, *infra*, three variants were cloned and characterized. Amino acid sequence comparison of these OB-R variants with a published human OB-R sequence 35 (Tartaglia et al., 1995, Cell 83:1263-1271) reveals three amino acid differences in their extracellular domains and extensive diversity in their intracellular cytoplasmic

domains. More specifically, Figure 1A-1E shows that in the variants, nucleotide residues #349-351 encode alanine, nucleotide residues #421-423 encode arginine and nucleotide residues #763-765 encode arginine. Additionally, the 5 variants diverge both in length and sequence composition from the human OB-R sequence published by Tartaglia et al. from nucleotide residue #2770 and beyond (Figure 2). In this regard, the intracellular domain of Form 1 (Figure 1A-1E) of the variants is highly homologous to a retrotransposon 10 sequence (Ono et al., 1987, *Nucl. Acid. Res.* 15:8725-8737). Such variants represent functionally defective forms of human OB-R in signal transduction upon leptin binding.

In order to clone additional variant forms of the molecule, labeled DNA probes made from nucleic acid fragments 15 corresponding to any portion of the cDNA disclosed herein may be used to screen a cDNA library prepared from human ovary, human prostate, human fetal liver, human lung, human kidney, human choroid plexus and human hypothalamus. More specifically, oligonucleotides corresponding to either the 5' 20 or 3' terminus of the cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 25 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in 30 neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon 35 sperm DNA at 0.5 mg/ml for 6 hours at 60°C. The radiolabelled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution

containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M EDTA) twice for 5 minutes each at room temperature, then in 5 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is aligned with the filters to select a positive plaque. If a 10 single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCl, pH 7.5, 0.01% gelatin. The phage may then be replated and rescreened to obtain single, well 15 isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

One method for identifying all 3' isoforms is to PCR 20 amplify the 3' ends of the variant cDNA from a variety of tissues including but not limiting to, choroid plexus, hypothalamus, fetal liver, bone marrow, ovary, or prostate. To obtain the 3' end of the cDNA, an oligo-dT primer is used 25 to synthesize the cDNA first strand. OB-R specific primers from the conserved region of the gene (e.g. up stream of nucleotide 2770) and oligo-dT are then used to amplify the 3' end. The PCR fragments are cloned and sequenced by standard techniques. Once obtained, these sequences may be translated 30 into amino acid sequence and examined for certain landmarks such as continuous open reading frame, regulatory regions that associate with tyrosine kinase activation, and finally overall structural similarity to known OB-R variants. These 3' variants may represent additional signal transduction defective forms of OB-R.

### 5.2. EXPRESSION OF THE OB-R VARIANTS

In accordance with the invention, the OB-R variant polynucleotide sequence which encodes a protein, peptide fragments, fusion proteins or functional equivalents thereof, 5 may be used to generate recombinant DNA molecules that direct the expression of the protein, peptide fragments, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a 10 part of such polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a 15 functionally equivalent amino acid sequence, may be used in the practice of the invention for the expression of the OB-R variant. Such DNA sequences include those which are capable of hybridizing to the OB-R variant sequence under stringent conditions, particularly at its 3' end. The phrase 20 "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, 25 for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at PH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, 30 sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or 35 substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions,

additions or substitutions of amino acid residues within the OB-R variant sequence, which result in a silent change thus producing a functionally equivalent protein. Such amino acid substitutions may be made on the basis of similarity in 5 polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged 10 polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

15 The DNA sequence of the invention may be engineered in order to alter the OB-R variant coding sequence for a variety of ends, including but not limited to, alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which 20 are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. In addition, the intracellular domain may also be altered and replaced by a different domain, such as the OB-R intracellular domain by 25 Tartaglia et al.

In another embodiment of the invention, the OB-R variant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or stimulators of receptor activity, 30 it may be useful to encode a chimeric protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the OB-R variant sequence and the heterologous protein sequence, so that the 35 variant may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of the OB-R variant could be synthesized in whole or

in part, using chemical methods well known in the art. (See, for example, Caruthers et al., 1980, *Nuc. Acids Res. Symp. Ser.* 7:215-233; Crea and Horn, 1980, *Nuc. Acids Res.* 9(10):2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters* 21:719; and Chow and Kempe, 1981, *Nuc. Acids Res.* 9(12):2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize OB-R variant amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, 10 cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, *Proteins Structures And Molecular Principles*, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or 15 sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

In order to express the OB-R variant in host cells, the nucleotide sequence coding for the variant, or a functional 20 equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. The expressed gene products as well as host cells or cell lines transfected or transformed with recombinant OB- 25 R variant expression vectors can be used for a variety of purposes. For example, host cells expressing the OB-R variant may be used to verify the ability of this molecule to bind leptin in a binding assay with radiolabeled, enzyme-conjugated or fluorescent dye-conjugated leptin. At the same 30 time, the ability of the molecule to transduce an activation signal in host cells upon binding to leptin may be tested by assaying proliferation or phosphorylation pattern of kinases in the cells. In addition, genetically-engineered host cells can be used to screen for and select agonist and antagonist 35 compounds, including any inhibitors that would interfere with binding of leptin to the extracellular or intracellular domain of the OB-R variant. In that connection, such host

cells may be used to screen for and select small molecules i.e., peptides, nucleic acids and synthetic compounds that can supplement the incomplete signal transduced by the OB-R variant following leptin binding. Such small molecules may 5 also affect receptor isoform pairing, thereby modifying the ability of OB-R to respond to leptin. Other uses, include, but are not limited to generating antibodies (i.e., monoclonal or polyclonal) that competitively inhibit activity of an OB-R variant, neutralize its activity, or even enhances 10 it activity. Antibodies may be used in detecting and quantifying expression of OB-R levels in cells and tissues.

### 5.3. USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be used for 15 diagnostic and/or therapeutic purposes. For diagnostic purposes, an OB-R variant polynucleotide may be used to detect gene expression or aberrant gene expression in infertile individuals as well as in normal individuals to identify predisposition for infertility. Included in the 20 scope of the invention are oligonucleotide sequences, that include antisense RNA and DNA molecules, ribozymes and triplex DNA, that function to inhibit translation of OB-R variant.

#### 25 5.3.1. DIAGNOSTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may have a number of uses for the diagnosis of the possible causes underlying infertility, resulting from expression of a defective receptor variant. For example, the OB-R variant cytoplasmic 30 domain DNA sequence may be used in hybridization assays of biopsy or autopsy materials obtained from ovary or prostate to diagnose OB-R variant expression; e.g., Southern or Northern analysis, including *in situ* hybridization assays as well as PCR. Such techniques are well known in the art, and 35 are in fact the basis of many commercially available diagnostic kits. For PCR detection, primers may be designed from a conserved region of the coding sequence and within the

3' region of OB-R variant. The tissues suitable for such analysis include but are not limited to, prostate, ovary, and testes, ova, sperm (semen), and cells in the ovarian follicular fluids.

5

### 5.3.2. THERAPEUTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be useful in the treatment of infertile conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not respond to leptin normally due to expression of a defective OB-R variant. In some instances, the polynucleotide encoding a functional OB-R is intended to replace or act in the place of the functionally defective OB-R variant gene. Alternatively, abnormal conditions characterized by expression of two copies of the OB-R variant can be treated using the gene therapy techniques described below.

Non-responsiveness to normal levels of leptin may contribute to infertility. This may result from a functionally defective receptor that does not transduce competent signals upon ligand binding. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express signalling competent forms of OB-R which may be used to augment the non-responsiveness of the naturally occurring OB-R variant. A signalling competent form may be, for example, a protein with the same extracellular domain and transmembrane region, but containing all or part of its normal signal transduction domain, such as that described by Tartaglia et al. (1995, Cell 83:1263-1271). Thus, recombinant gene therapy vectors may be used therapeutically for treatment of infertility resulting from expression or activity of the OB-R variant. Accordingly, the invention provides a method of augmenting signal transduction by an endogenous OB-R variant in a cell comprising delivering a DNA molecule encoding a signalling competent form of the OB-R to the cell so that the signalling competent protein is produced

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in the cell and competes with the endogenous defective OB-R variant for access to molecules in the signalling pathway which does not activate or are not activated by the endogenous natural defective receptor. Additionally, since 5 dimerization of a functional receptor with a defective variant may occur in cells of heterozygous individuals, small molecules may be used to inhibit such pairing, thereby increasing the number of functional dimeric receptors for proper signalling in response to leptin.

10 Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant functional OB-R into the targeted cell population. Methods which are well known to those skilled in 15 the art can be used to construct recombinant viral vectors containing an OB-R polynucleotide sequence. See, for example, the techniques described in Sambrook et al., 1989, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant OB-R molecules 20 can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences including anti-sense RNA and 25 DNA molecules and ribozymes that function to inhibit the translation of the OB-R variant mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to 30 antisense DNA, oligodeoxyribonucleotides derived from the OB-R variant nucleotide sequence at nucleotide #2771 and beyond, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of 35 ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the

invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of OB-R variant RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Oligodeoxyribonucleotides can form sequence-specific triple helices by hydrogen bonding to specific complementary sequences in duplexed DNA. Interest in triple helices has focused on the potential biological and therapeutic applications of these structures. Formation of specific triple helices may selectively inhibit the replication and/or gene expression of targeted genes by prohibiting the specific binding of functional trans-acting factors.

Oligonucleotides to be used in triplex helix formation should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Oligonucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich oligonucleotides provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, oligonucleotides may be chosen that are purine-rich, for example, containing a stretch of G residues. These

oligonucleotides will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in 5 the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" oligonucleotide.

Switchback oligonucleotides are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one 10 strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art 15 for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding 20 the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or 25 inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not 30 limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

#### 5.4. ACTIVATION OF TYROSINE KINASE PATHWAYS IN INFERTILITY

Many known class I cytokine receptors initiate cell signaling via Janus kinases (JAKs) (Ihle, 1995, *Nature* 377:591-594; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., 1994, *Cell* 76:253-62; Ziemiecki et al., 1994, *Trends Cell. Biol.* 4:207-212). JAK1-3 have been shown to bind to conserved sequences termed box1 and box2 (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53). Ligand binding induces a homo- or hetero-dimerization of receptor chains which activates, by phosphorylation, the JAKs. The activated JAKs, in turn, phosphorylate members of the STAT family (Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., *Blood* 86:1243-54; Darnell et al., 1994, *Science* 264:1415-21; Zhong et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4806-10; Hou et al., 1994, *Science* 265:1701-6). These phosphorylated STATs ultimately translocate to the nucleus, form transcription complexes, and regulate gene expression. Both box1 and box2 are required for complete signaling in certain systems. (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53).

The OB-R variants disclosed herein have a typical box1 (from nucleotide #2707-2730) that contains the critical xWxxxPxP amino acid sequence, but they do not contain an obvious box2 nor further downstream sequences that are important for normal receptor activation. Therefore, it is possible to use compounds that activate JAKs to directly activate these pathways for improving fertility without triggering the OB-R.

#### 6. EXAMPLE: MOLECULAR CLONING OF OB-R VARIANTS

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (designated as Hu-B1.219 #4, #33, #34, #1, #3, #8, #36, #55, #57, #60 and #62) contained overlapping sequences,

which were then compiled into a contiguous nucleotide sequence referred to as Hu-B1.219. When the deduced amino acid sequence of one such sequence (Figure 1A-1E) was compared with the sequence of a recently published human OB-R (Tartaglia et al., 1995, Cell 83:1263), they were shown to be nearly identical in the extracellular domains with the exception of three amino acids, whereas there existed extensive diversity in their intracellular cytoplasmic domains at the 3' end. This sequence encodes an OB-R variant 5 herein referred to as Form 1. In addition, two other variants were identified, and they both differ from the human OB-R published by Tartaglia et al (1995, Cell 83:1263) in their 3' ends (Figure 2). These two additional variants are referred to as Forms 2 and 3 of OB-R. The predicted protein 10 sequences of the variants (Figure 3A-3C) contain two FN III domains, each containing a "WS box", which are characteristic 15 of genes of the Class I cytokine receptor family.

When various human tissue RNA were probed with a fragment containing a sequence commonly shared by the OB-R 20 variants by Northern blot analysis, expression was detected in heart, placenta, lung, liver, muscle, pancreas, prostate, ovary, small intestine and brain (Table I).

Based on the sequence presented in Figure 1A-1E, the translation initiation site appears at position #97. The 25 sequences of Forms 1, 2 and 3 encode an open reading frame up to and including nucleotide #2970, #2814 and #2784 (Figure 2), respectively. It is believed that the sequence between nucleotides #2629 and #2682 (Figure 1A-1E) encodes a transmembrane domain. The complete sequences of Forms 1, 2 30 and 3 encode proteins of 958, 906 and 896 amino acids, respectively.

TABLE I

SUMMARY OF NORTHERN BLOT ANALYSIS OF  
OB-R EXPRESSION IN HUMAN TISSUES AND CELL LINES

5

Developmental Stage	Tissue Type	Expression
10	fetal brain	-
	fetal lung	+++
	fetal liver	+++++
	fetal kidney	+
15	adult heart	++
	adult brain	+/-
	adult placenta	+
	adult lung	+
	adult liver	+++
	adult skeletal muscle	+
	adult kidney	+/-
	adult pancreas	+
	adult spleen	+/-
	adult thymus	+/-
20	adult prostate	++
	adult testis	+/-
	adult ovary	+++
	adult small intestine	++
	adult colon	-
	adult peripheral blood	-
30	adult leukocytes	-

35

17

Developmental Stage	Tissue Type	Expression
5 cancer	HL-60	-
	HeLa	-
	K-562	+++
	MOLT-4	-
	Raji	-
	SW480	-
10	A549	+
	G361	-

The sequences of the three OB-R variants are identical to the sequence of human OB-R reported by Tartaglia (1995, 15 Cell 83:1263-1271) in the transmembrane region and a portion of the intracellular domain up to and including nucleotide #2769 (Figure 2), then they diverge at nucleotide #2770 and beyond. In addition, the products of these cDNA are substantially shorter in their intracellular domain than the 20 human OB-R published by Tartaglia et al. These isoforms of OB-R may derive from a common precursor mRNA by an alternative splicing mechanism. The sequence in this region is consistent with well known splice junctions. It is noteworthy that the DNA sequence of Form 1 (Figure 1A-1E) of 25 the OB-R variant from nucleotide #2768 to the end is 98% identical to a human retrotransposon sequence that is thought to be derived from a human endogenous retroviral DNA sequence (Singer, 1982, Cell 28:433; Weiner et al., 1986, Ann. Rev. Biochem. 55:631; Lower et al., 1993, Proc. Natl. Acad. Sci. 30 USA 90:4480; Ono et al., 1987, Nucl. Acid. Res. 15:8725- 8735).

7. EXAMPLE: EXPRESSION OF OB-R IN SEX HORMONE PRODUCING CELLS IN THE OVARY AND DETECTION OF LEPTIN IN OVARIAN FOLLICULAR FLUIDS

7.1. MATERIALS AND METHODS

5

7.1.1. REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION (RT/PCR)

Total RNA was isolated using standard laboratory procedures (Sambrook et al., 1989, Molecular Cloning, A 10 Laboratory Manual, Cold Spring Harbor Laboratory, NY). Approximately 1  $\mu$ g of total RNA was reverse transcribed and the cDNA was amplified by PCR (Perkin Elmer, Norwalk, CT). The PCR amplification conditions were: 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec for a total of 40 cycles. The 15 amplified products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The OB-R amplimers were GGTTTGCATATGGAAGTC (upper) and CCTGAACCATCCAGTCTCT (lower). The Form 1 specific amplimers were GACTCATTGTGCAGTGTTCA (upper) and TAGTGGAGGGAGGGTCAGCAG 20 (lower).

7.2. RESULTS

Table I in Section 6, *supra*, shows that the OB-R is expressed in reproductive organs such as ovary and prostate 25 gland. In order to determine the specific cell types in the ovary that expressed the receptor, primary granulosa and cumulus cell cultures were established from the ovaries and assayed for OB-R expression by RT/PCR. In Table II, the cells that produced sex hormones in the ovary, i.e. granulosa 30 and cumulus cells, expressed the different forms of OB-R.

TABLE II

## OB-R EXPRESSION IN GRANULOSA AND CUMULUS CELLS BY RT/PCR

5	Cell Types	Form 1	Form 3	OB-R*
	Granulosa cells derived from ovarian follicles	+	+	+-
10	Granulosa cells derived from ovarian follicles	-	+	+
	Cumulus cells derived from oocytes	+	+	+

\* OB-R refers to the published sequence by Tartaglia (1995, Cell 83:1263-1271).

15 Additionally, ovarian follicular fluids were obtained from several patients and assayed for the presence of leptin. The detection assay utilized an interleukin-3-dependent cell line, BaF3, that had been transfected with a chimeric receptor construct containing the extracellular domain of murine OB-R ligated to the transmembrane and cytoplasmic 20 domains of the thrombopoietin receptor. Both the BaF3 parental cell line and the transfected cell line responded to IL-3, whereas only the transfected cells responded to leptin (Figure 4).

25 When the cells were incubated with follicular fluids, the transfected cell line was induced to proliferate as compared with the parental cell line as a control (Figure 4). The cell growth-stimulating activity in the fluids was shown to be leptin since the activity was specifically inhibited by the addition of soluble murine OB-R (Figure 5). Therefore, 30 leptin is present in the follicular fluids, and it stimulates OB-R-expressing cells in the ovary to proliferate.

8. DEPOSIT OF MICROORGANISMS

35 The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

Strain Designation Accession No.

HuB1.219, #1	75885
HuB1.219, #4	75886
HuB1.219, #8	75887
HuB1.219, #33	75888
5 HuB1.219, #34	75889
HuB1.219, #36	75890
HuB1.219, #55	75971
HuB1.219, #60	75973
HuB1.219, #3	75970
HuB1.219, #57	75972
HuB1.219, #62	75974

10 The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of individual aspects of the invention. Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to 15 those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

20 All publications cited herein are incorporated by reference in their entirety.

25

30

35

21

International Application No: PCT/

**MICROORGANISMS**Optional Sheet in connection with the microorganism referred to on page 36, lines 25-37 of the description**A. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

American Type Culture Collection

Address of depositary institution (including postal code and country)

12301 Parklawn Drive  
Rockville, MD 20852  
USDate of deposit September 14, 1994 Accession Number 75885**B. ADDITIONAL INDICATIONS** (leave blank if not applicable). This information is contained on a separate attached sheet**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (leave blank if not applicable)**D. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later. (Specify the general nature of the indications e.g., "Accession Number of Deposit")

**E.**  This sheet was received with the International application when filed (to be checked by the receiving Office)

(Authorized Officer)

 The date of receipt (from the applicant) by the International Bureau

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive  
Rockville, MD 20852  
US

<u>Accession No.</u>	<u>Date of Deposit</u>
75886	September 14, 1994
75887	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75890	September 14, 1994
75970	December 14, 1994
75971	December 14, 1994
75972	December 14, 1994
75973	December 14, 1994
75974	December 14, 1994

WHAT IS CLAIMED IS:

1. A method for detecting a defective OB-R in cells comprising:

- 5 (a) extracting RNA from a cell population;
- (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 1 in Figure 2; and
- (c) detecting hybridization of the RNA with the oligonucleotide.

10

2. The method of Claim 1 in which the cell population is obtained from ovary.

15 3. The method of Claim 1 in which the cell population is obtained from prostate.

4. The method of Claim 1 in which the cell population is obtained from testis.

20 5. The method of Claim 1 in which the cell population is obtained from sperm.

6. The method of Claim 1 in which the cell population is obtained from ovum.

25

7. The method of Claim 1 in which the cell population is obtained from cells of ovarian follicular fluids.

30 8. The method of Claim 1 in which the cell population is obtained from blood.

9. A method for detecting a defective OB-R in cells comprising:

- 35 (a) extracting RNA from a cell population;
- (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 2 in Figure 2; and

(c) detecting hybridization of the RNA with the oligonucleotide.

10. The method of Claim 9 in which the cell population 5 is obtained from ovary.

11. The method of Claim 9 in which the cell population is obtained from prostate.

10 12. The method of Claim 9 in which the cell population is obtained from testis.

13. The method of Claim 9 in which the cell population is obtained from sperm.

15

14. The method of Claim 9 in which the cell population is obtained from ovum.

15. The method of Claim 9 in which the cell population 20 is obtained from cells of ovarian follicular fluids.

16. The method of Claim 9 in which the cell population is obtained from blood.

25 17. A method for detecting a defective OB-R in cells comprising:

- (a) extracting RNA from a cell population;
- (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 3 in Figure 2; and
- (c) detecting hybridization of the RNA with the oligonucleotide.

30 18. The method of Claim 17 in which the cell population 35 is obtained from ovary.

19. The method of Claim 17 in which the cell population is obtained from prostate.

20. The method of Claim 17 in which the cell population is obtained from testis.

21. The method of Claim 17 in which the cell population is obtained from sperm.

10 22. The method of Claim 17 in which the cell population is obtained from ovum.

23. The method of Claim 17 in which the cell population is obtained from cells of ovarian follicular fluids.

15

24. The method of Claim 17 in which the cell population is obtained from blood.

25. A method for treating infertility, comprising  
20 administering to an individual an effective amount of an agent capable of inhibiting expression of an OB-R variant gene.

26. The method of Claim 25 in which the OB-R variant gene further comprises the sequence of Figure 2 or which is  
25 capable of selectively hybridizing to it.

27. The method of Claim 26 in which the agent is an antisense molecule complementary to mRNA encoded by the sequence of Figure 2.

30

28. The method of Claim 26 in which the agent is a ribozyme molecule specific for mRNA enclosed by the sequence of Figure 2.

35

29. The method of Claim 26 in which the agent is a triple helix component.

30. A method for identifying a compound capable of supplementing biological activity of leptin on cells of reproductive organs, comprising:

- 5 (a) incubating the cells expressing an OB-R variant with leptin;
- (b) incubating a portion of the leptin-treated cells with a test compound; and
- (c) comparing activation signal in the cells treated in step (b) with cells treated in step

10 (a);

thereby determining the compound that augments activation of the OB-R variant by leptin.

31. The method of Claim 30 in which the OB-R variant is encoded by the sequence depicted in Figure 2.

32. A method for identifying a compound capable of inhibiting biological activity of leptin on cells of reproductive organs, comprising:

- 20 (a) incubating the cells expressing an OB-R variant with leptin;
- (b) incubating a portion of the leptin-treated cells with a test compound; and
- (c) comparing activation signal in the cells treated in step (b) with cells treated in step

25 (a);

thereby determining the compound that inhibits activation of the OB-R variant by leptin.

30 33. The method of Claim 32 in which the OB-R variant is encoded by the sequence depicted in Figure 2.

35

GCG CGC GCG 9 ACG CAG 18 GTG CCC GAG 27 CGG CCC GCG CCC 36 ATC TCT 45 GCC TTC 54 GGT  
 A R A T Q V P E P R P A P I S A F G  
 CGA GTT GGA 63 CCC CCG GAT 72 CAA CGT 81 GTA CTT CTC TGA 90 AGT AAG ATG 99 ATT TGT 108 CAA  
 R V G P R D Q G V L L \* S K M I C Q  
 AAA TTC 117 TGT GTG GTT TTG TTA 126 CAT TGG 135 GAA TTT 144 ATT TAT GTG 153 ATA ACT 162 GCG TTT  
 K F C V V L L H W E F I Y V I T A F  
 AAC TTG 171 TCA TAT CCA ATT ACT CCT 180 TGG AGA 189 TTT AAG TTG TCT 198 TGC ATG 207 CCA CCA 216  
 N L S Y P I T P W R F K L S C M P P  
 AAT TCA 225 ACC TAT GAC TAC 234 TTC CTT 243 TTG CCT GCT GGA 252 CTC TCA AAG 261 AAT ACT 270 TCA  
 N S T Y D Y F L L P A G L S K N T S  
 AAT TCG 279 AAT GGA CAT 288 TAT GAG ACA 297 GCT GTT GAA CCT 306 AAG TTT AAT 315 TCA AGT 324 GGT  
 N S N G H Y E T A V E P K F N S S G  
 ACT CAC 333 TTT TCT AAC 342 TCC AAA GCA ACT 351 TTC CAC TGT TGC 360 TTT CGG AGT 378 GAG  
 T H F S N L S K A T F H C C F R S E  
 CAA GAT 387 AGA AAC 396 TGC TCC 405 TTA TGT GCA GAC 414 AAC ATT GAA GGA 423 AGG ACA 432 TTT GTT  
 Q D R N C S L C A D N I E G R T F V  
 TCA ACA 441 GTA AAT 450 TCT TTA GTT 459 TTT CAA CAA ATA GAT 468 GCA AAC TGG 477 AAC ATA 486 CAG  
 S T V N S L V F Q Q I D A N W N I Q  
 TGC TGG 495 CTA AAA 504 GGA GAC 513 TTA AAA TTA TTC 522 ATC TGT TAT GTG 531 GAG TCA 540 TTA TTT  
 C W L K G D L K L F I C Y V E S L F  
 AAG AAT 549 CTA TTC 558 AGG AAT TAT AAC TAT 567 AAG GTC 576 CAT CTT TTA TAT 585 GTT CTG 594 CCT  
 K N L F R N Y N Y K V H L L Y V L P  
 GAA GTG 603 TTA GAA GAT 612 TCA CCT 621 CTG GTT CCC CAA AAA GGC AGT 630 TTT 639 CAG ATG 648 GTT  
 E V L E D S P L V P Q K G S F Q M V

Figure 1A

657	666	675	684	693	702
CAC TGC AAT TGC AGT GTT CAT GAA	TGT TGT GAA TGT CTT GTG CCT GTG CCA AGA				
H C N C S V H E C C E C L V P V P T					
711	720	729	738	747	756
GCC AAA CTC AAC GAC ACT CTC CTT ATG TGT TTG AAA ATC ACA TCT GGT GGA GTC					
A K L N D T L L M C L K I T S G G V					
765	774	783	792	801	810
ATT TTC CGG TCA CCT CTA ATG TCA GTT CAG CCC ATA AAT ATG GTG AAG CCT CAT					
I F R S P L M S V Q P I N M V K P D					
819	828	837	846	855	864
CCA CCA TTA GGT TTG CAT ATG GAA ATC ACA GAT GAT GGT AAT TTA AAG ATT TCT					
P P L G L H M E I T D D G N L K I S					
873	882	891	900	909	918
TGG TCC AGC CCA CGA TTG GTA CCA TTT CCA CTT CAA TAT CAA GTG AAA TAT TCA					
W S S P P L V P F P L Q Y Q V K Y S					
927	936	945	954	963	972
GAG AAT TCT ACA ACA GGT ATC AGA GAA GCT GAC AAG ATT GTC TCA GCT ACA TCC					
E N S T T V I R E A D K I V S A T S					
981	990	999	1008	1017	1026
CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT GAG GTT CAG GTG AGG GGC					
L L V D S I L P G S S Y E V Q V R G					
1035	1044	1053	1062	1071	1080
AAG AGA CTG GAT GCC CCA GGA ATC TGG AGT GAC TGG AGT ACT CCT CGT GTC TTT					
K R L D G P G I W S D W S T P R V F					
1089	1098	1107	1116	1125	1134
ACC ACA CAA GAT GTC ATA TAC TTT CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT					
T T Q D V I Y F P P K I L T S V G S					
1143	1152	1161	1170	1179	1188
AAT GTT TCT TTT CAC TGC ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA					
N V S F H C I Y K K E N K I V P S K					
1197	1206	1215	1224	1233	1242
GAG ATT GTT TGG TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT					
E I V W W M N L A E K I P Q S Q Y D					
1251	1260	1269	1278	1287	1296
GTT GTG AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA					
V V S D H V S K V T F F N L N E T K					
1305	1314	1323	1332	1341	1350
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT GAA TCC					
P R G K F T Y D A V Y C C N E H E C					

Figure 1B

1359	1368	1377	1386	1395	1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT	-----	-----	-----	ATC TCA TGT	-----
H H R Y A E L Y V I D V N I N I S C	-----	-----	-----	-----	-----
1413	1422	1431	1440	1449	1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC	-----	-----	-----	AGT ACA ATC	-----
E T D G Y L T K M T C R W S T S T I	-----	-----	-----	-----	-----
1467	1476	1485	1494	1503	1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC	-----	-----	-----	AGC CTT TAC	-----
Q S L A E S T L Q L R Y H R S S L Y	-----	-----	-----	-----	-----
1521	1530	1539	1548	1557	1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT	-----	-----	-----	TGC TAT TTG	-----
C S D I P S I H P I S E P K D C Y L	-----	-----	-----	-----	-----
1575	1584	1593	1602	1611	1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA	-----	-----	-----	TTA TCT GGC	-----
Q S D G F Y E C I F Q P I F L L S G	-----	-----	-----	-----	-----
1629	1638	1647	1656	1665	1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC	-----	-----	-----	TCT CCA CCA	-----
Y T M W I R I N H S L G S L D S P P	-----	-----	-----	-----	-----
1683	1692	1701	1710	1719	1728
ACA TGT GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC	-----	-----	-----	AGT GTG AAA	-----
T C V L P D S V V K P L P P S S V K	-----	-----	-----	-----	-----
1737	1746	1755	1764	1773	1782
GCA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA	-----	-----	-----	AAG CCA GTC	-----
A E I T I N I G L L K I S W E K P V	-----	-----	-----	-----	-----
1791	1800	1809	1818	1827	1836
TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA AGT	-----	-----	-----	GGA AAA GAA	=
F P E N N L Q F Q I R Y G L S G K E	-----	-----	-----	-----	-----
1845	1854	1863	1872	1881	1890
GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT GCA AAA TCA AAA TCT	-----	-----	-----	GTC AGT CTC	-----
V Q W K M Y E V Y D A K S K S V S L	-----	-----	-----	-----	-----
1899	1908	1917	1926	1935	1944
CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT GTT CAG GTG CGC TGT	-----	-----	-----	AAG AGG CTA	-----
P V P D L C A V Y A V Q V R C K R L	-----	-----	-----	-----	-----
1953	1962	1971	1980	1989	1998
GAT GGA CTG GGA TAT TGG AGT AAT TGG ACC AAT CCA GCC TAC ACA	-----	-----	-----	GTG GTC ATG	-----
D G L G Y W S N W S N P A Y T V V M	-----	-----	-----	-----	-----
2007	2016	2025	2034	2043	2052
GAT ATA AAA GTT CCT ATG AGA GGA CCT GAA TTT TGG AGA ATA ATT	-----	-----	-----	AAT GGA CAT	-----
D I K V P M R G P E F W R I I N G D	-----	-----	-----	-----	-----

Figure 1C

2051	2070	2079	2088	2097	2106
ACT ATG AAA	AAG GAG AAA AAT GTC ACT	TTA CTT TGG AAG CCC	CTG ATG AAA AAT		
T M K	K E K N V T L L W K P L M K N				
2115	2124	2133	2142	2151	2160
GAC TCA TTG	TGC AGT GTT CAG AGA TAT GTG ATA AAC	CAT CAT ACT	TCC TGC AAT		
D S L	C S V Q R Y V I N H H T S C N				
2169	2178	2187	2196	2205	2214
GGA ACA TGG	TCA GAA GAT GTG GGA AAT CAC ACG AAA	TTC ACT TTC	CTG TGG ACA		
G T W S E D V G N H T K F T E L W T					
2223	2232	2241	2250	2259	2268
GAG CAA GCA	CAT ACT GTT ACG GTT CTG GCC	ATC AAT TCA ATT GGT	GCT TCT GTT		
E Q A H T V T V L A I N S I G A S V					
2277	2286	2295	2304	2313	2322
GCA AAT TTT AAT	TTA ACC TTT TCA TGG CCT ATG AGC AAA	GTA AAT ATC	GTG CAG		
A N F N L T F S W P M S K V N I V Q					
2331	2340	2349	2358	2367	2376
TCA CTC AGT GCT	TAT CCT TTA AAC AGC AGT TGT	GTG ATT GTT TCC	TGG ATA CTA		
S L S A Y P L N S S C V I V S W I L					
2385	2394	2403	2412	2421	2430
TCA CCC AGT GAT	TAC AAG CTA ATG TAT	TTT ATT ATT GAG TGG AAA	AAT CTT AAT		
S P S D Y K L M Y F I I E W K N L N					
2439	2448	2457	2466	2475	2484
GAA GAT GGT	GAA ATA AAA TGG CTT AGA ATC TCT	TCA TCT GTT AAG	AAG TAT TAT		
E D G E I K W L R I S S S V K K Y Y					
2493	2502	2511	2520	2529	2538
ATC CAT GAT	CAT TTT ATC CCC ATT GAG AAG TAC	CAG TTC AGT CTT	TAC CCA ATA		
I H D H F I P I E K Y Q F S L Y P I					
2547	2556	2565	2574	2583	2592
TTT ATG GAA GGA GTG GGA AAA CCA AAG ATA ATT AAT AGT	TTC ACT CAA GAT GAT				
F M E G V G K P K I I N S F T Q D D					
2601	2610	2619	2628	2637	2646
ATT GAA AAA CAC CAG AGT GAT GCA GGT	TTA TAT GTA ATT GTG CCA GTC	ATT ATT			
I E K H Q S D A G L Y V I V P V I I					
2655	2664	2673	2682	2691	2700
TCC TCT TCC	ATC TTA TTG CTT GGA ACA TTA TTA ATA TCA CAC CAA	AGA ATG AAA			
S S S I L L L G T L L I S H Q R M K					
2709	2718	2727	2736	2745	2754
AAG CTA TTT TGG GAA GAT GTT CCG AAC CCC	AAC AAG AAT TGT TCC TGG CCA CAA CGA				
K L F W E D V P N P K N C S W A Q G					

Figure 1D

2763	2772	2781	2790	2799	2808
CTT AAT TTT CAG AAG ATG CTT GAA GGC AGC ATG TTC GTT AAG AGT CAT CAC CAC					
L N F Q K M L E G S M F V K S H H H					
2817	2826	2835	2844	2853	2862
TCC CTA ATC TCA AGT ACC CAG GGA CAC AAA CAC TGC GGA AGG CCA CAG GGT CCT					
S L I S S T Q G H K H C G R P Q G P					
2871	2880	2889	2898	2907	2916
CTG CAT AGG AAA ACC AGA GAC CTT TGT TCA CTT GTT TAT CTG CTG ACC CTC CCT					
L H R K T R D L C S L V Y L L T L P					
2925	2934	2943	2952	2961	2970
CCA CTA TTG TCC TAT GAC CCT GGC AAA TCC CCC TCT GTG AGA AAC ACC CAA GAA					
P L L S Y D P A K S P S V R N T Q E					
2979	2988				
TGA TCA ATA AAA AAA AAA AAA 3'					
* S I K K K K					

Figure 1 E

	2760	2770	2780	2790	2800	
Form 1	2751	AGGACTTAAT	TTTCAGAAGA	TCCTTGAAGG	CAGCACTGTC	GTTAAGAGTC
2	2751	AGGACTTAAT	TTTCAGAAGA	AAATGCCCTGG	CACAAAGGAA	CTACTGGGTG
3	2751	AGGACTTAAT	TTTCAGAAGA	CAACGGACAT	TCTTTGAAGT	CTAACATCA
	2800	2800	2800	2800	2800	
	2810	2820	2830	2840	2850	
Form 1	2801	ATCACCACTC	CCTAACTCTA	AGTACCCAGG	GACACAAACA	CTGGGGAAGG
2	2801	GAGGTGGTT	GACTTAGGAA	ATGCTTGTGA	AGCTACGTC	TACCTCGTGC
3	2801	TCACATACGA	TGAACTTCAAT	GTGCCAACTT	CCAAACAGTC	TATAGAGTAT
	2850	2850	2850	2850	2850	
	2860	2870	2880	2890	2900	
Form 1	2851	CCACAGGGTC	CTCTGCATAG	GAAACCAGA	GACCTTTGTT	CACTTGTATA
2	2851	GCACCTGCTC	TCCCTGAGGT	GTGCCAAATG	.....	.....
3	2851	TAGAAGTTT	TTACATTCTG	AAGAAGG	.....	.....
	2900	2900	2900	2900	2900	
	2910	2920	2930	2940	2950	
Form 1	2901	TCTGGTGACC	CTCCCTCAC	TATTCCTCTA	TGACCCCTGCC	AAATCCCCCT
2	2901	.....	.....	.....	.....	.....
3	2901	.....	.....	.....	.....	.....
	2950	2950	2950	2950	2950	
	2960	2970	2980	2990	3000	
Form 1	2951	CTGTGAGAA	CACCCAAAGA	TGATCAATAA	AAAAAAAGAA	A.....
2	2951	.....	.....	.....	.....	.....
3	2951	.....	.....	.....	.....	.....
	3000	3000	3000	3000	3000	

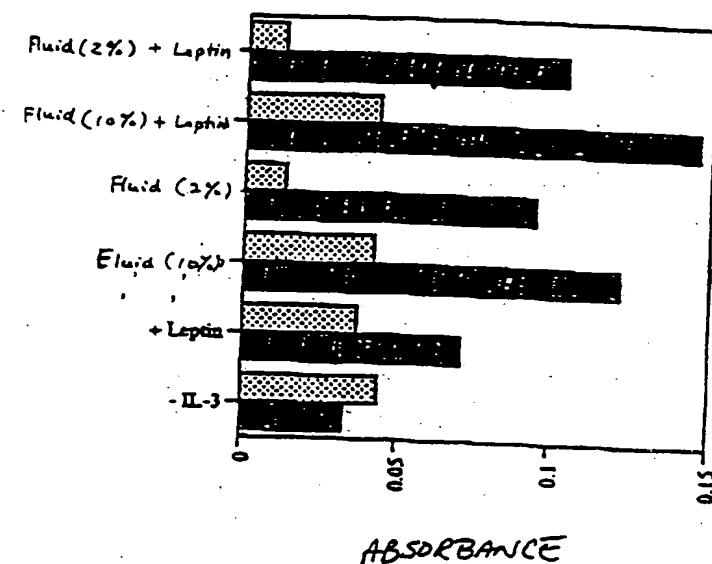
Figure 2.

Figure 3A

HuB1.219_1	451	TSTGDSRSES	460	TGQRYHRS	470	LYGSDEPSIH	480	PISEPKDCYI	490	QSDGAEYCIB	500
HuB1.219_2	451	TSTGDSRSES	451	TGQRYHRS	451	LYGSDEPSIH	451	PISEPKDCYI	451	QSDGAEYCIB	500
HuB1.219_3	451	TSTGDSRSES	451	TGQRYHRS	451	LYGSDEPSIH	451	PISEPKDCYI	451	QSDGAEYCIB	500
HuOBR	451	TSTGDSRSES	451	TGQRYHRS	451	LYGSDEPSIH	451	PISEPKDCYI	451	QSDGAEYCIB	500
MuOBR	451	PSHDSAVGS	451	TGQRYHRS	451	LYGSDEPSIH	451	PISEPKDCYI	451	QSDGAEYCIB	500
										QSDGAEYCIB	500
HuB1.219_1	501	QPTHDSGIV	510	MWIRGHRSG	520	SLFGPPETIV	530	PSDVAKPDPEP	540	SSUGETIV	550
HuB1.219_2	501	QPTHDSGIV	501	MWIRGHRSG	501	SLFGPPETIV	501	PSDVAKPDPEP	501	SSUGETIV	550
HuB1.219_3	501	QPTHDSGIV	501	MWIRGHRSG	501	SLFGPPETIV	501	PSDVAKPDPEP	501	SSUGETIV	550
HuOBR	501	QPTHDSGIV	501	MWIRGHRSG	501	SLFGPPETIV	501	PSDVAKPDPEP	501	SSUGETIV	550
MuOBR	501	QPTHDSGIV	501	MWIRGHRSG	501	SLFGPPETIV	501	PSDVAKPDPEP	501	SSUGETIV	550
										SSUGETIV	550
HuB1.219_1	551	TGQYKGSWIK	560	EVPEPENLOP	570	QIRYGLSGKE	580	VOVIMYEVAD	590	AKSYASLYV	600
HuB1.219_2	551	TGQYKGSWIK	551	EVPEPENLOP	551	QIRYGLSGKE	551	VOVIMYEVAD	551	AKSYASLYV	600
HuB1.219_3	551	TGQYKGSWIK	551	EVPEPENLOP	551	QIRYGLSGKE	551	VOVIMYEVAD	551	AKSYASLYV	600
HuOBR	551	TGQYKGSWIK	551	EVPEPENLOP	551	QIRYGLSGKE	551	VOVIMYEVAD	551	AKSYASLYV	600
MuOBR	551	TGQYKGSWIK	551	EVPEPENLOP	551	QIRYGLSGKE	551	VOVIMYEVAD	551	AKSYASLYV	600
										AKSYASLYV	600
HuB1.219_1	601	PDGKAVVAG	610	VRGKRCDDG	620	YNSANSNPAY	630	IVAMDIKVPA	640	PGPEPEHIN	650
HuB1.219_2	601	PDGKAVVAG	601	VRGKRCDDG	601	YNSANSNPAY	601	IVAMDIKVPA	601	PGPEPEHIN	650
HuB1.219_3	601	PDGKAVVAG	601	VRGKRCDDG	601	YNSANSNPAY	601	IVAMDIKVPA	601	PGPEPEHIN	650
HuOBR	601	PDGKAVVAG	601	VRGKRCDDG	601	YNSANSNPAY	601	IVAMDIKVPA	601	PGPEPEHIN	650
MuOBR	601	SPDGKAVVAG	601	VRGKRCDDG	601	YNSANSNPAY	601	IVAMDIKVPA	601	PGPEPEHIN	650
										PGPEPEHIN	650
HuB1.219_1	651	GTGKIGKGV	660	TEHKGKGV	670	DSKCSVORYV	680	INHHTSENGI	690	WSEPDVNTIK	700
HuB1.219_2	651	GTGKIGKGV	651	TEHKGKGV	651	DSKCSVORYV	651	INHHTSENGI	651	WSEPDVNTIK	700
HuB1.219_3	651	GTGKIGKGV	651	TEHKGKGV	651	DSKCSVORYV	651	INHHTSENGI	651	WSEPDVNTIK	700
HuOBR	651	GTGKIGKGV	651	TEHKGKGV	651	DSKCSVORYV	651	INHHTSENGI	651	WSEPDVNTIK	700
MuOBR	651	GTGKIGKGV	651	TEHKGKGV	651	DSKCSVORYV	651	INHHTSENGI	651	WSEPDVNTIK	700
										WSEPDVNTIK	700
HuB1.219_1	701	STEGKTVAH	710	TEVPAHNSI	720	GASVANENLT	730	FSWPMISKVNT	740	VOSLSAEPIN	750
HuB1.219_2	701	STEGKTVAH	701	TEVPAHNSI	701	GASVANENLT	701	FSWPMISKVNT	701	VOSLSAEPIN	750
HuB1.219_3	701	STEGKTVAH	701	TEVPAHNSI	701	GASVANENLT	701	FSWPMISKVNT	701	VOSLSAEPIN	750
HuOBR	701	STEGKTVAH	701	TEVPAHNSI	701	GASVANENLT	701	FSWPMISKVNT	701	VOSLSAEPIN	750
MuOBR	701	STEGKTVAH	701	TEVPAHNSI	701	GASVANENLT	701	FSWPMISKVNT	701	VOSLSAEPIN	750
										VOSLSAEPIN	750
HuB1.219_1	751	SSGKAVVAG	760	SPSDYKGGVY	770	TEHKNLNEED	780	GETKWLRISS	790	SVAKYVTHD	800
HuB1.219_2	751	SSGKAVVAG	751	SPSDYKGGVY	751	TEHKNLNEED	751	GETKWLRISS	751	SVAKYVTHD	800
HuB1.219_3	751	SSGKAVVAG	751	SPSDYKGGVY	751	TEHKNLNEED	751	GETKWLRISS	751	SVAKYVTHD	800
HuOBR	751	SSGKAVVAG	751	SPSDYKGGVY	751	TEHKNLNEED	751	GETKWLRISS	751	SVAKYVTHD	800
MuOBR	751	SSGKAVVAG	751	SPSDYKGGVY	751	TEHKNLNEED	751	GETKWLRISS	751	SVAKYVTHD	800
										SVAKYVTHD	800
HuB1.219_1	801	STEGKTVDS	810	TEVPAHNSI	820	KPKGKNSFTG	830	DDIEKHKOSPA	840	GLYVYVPIV	850
HuB1.219_2	801	STEGKTVDS	801	TEVPAHNSI	801	TEVPAHNSI	801	DDIEKHKOSPA	801	GLYVYVPIV	850
HuB1.219_3	801	STEGKTVDS	801	TEVPAHNSI	801	TEVPAHNSI	801	DDIEKHKOSPA	801	GLYVYVPIV	850
HuOBR	801	STEGKTVDS	801	TEVPAHNSI	801	TEVPAHNSI	801	DDIEKHKOSPA	801	GLYVYVPIV	850
MuOBR	801	STEGKTVDS	801	TEVPAHNSI	801	TEVPAHNSI	801	DDIEKHKOSPA	801	GLYVYVPIV	850
										GLYVYVPIV	850
HuB1.219_1	851	SSSXLGTV	860	LISHORMKGG	870	TEWDPENPKN	880	CSWAGGLNFO	890	KMLEGSFMVK	900
HuB1.219_2	851	SSSXLGTV	851	LISHORMKGG	851	TEWDPENPKN	851	CSWAGGLNFO	851	KMLEGSFMVK	900
HuB1.219_3	851	SSSXLGTV	851	LISHORMKGG	851	TEWDPENPKN	851	CSWAGGLNFO	851	KMLEGSFMVK	900
HuOBR	851	SSSXLGTV	851	LISHORMKGG	851	TEWDPENPKN	851	CSWAGGLNFO	851	KMLEGSFMVK	900
MuOBR	851	SSSXLGTV	851	LISHORMKGG	851	TEWDPENPKN	851	CSWAGGLNFO	851	KMLEGSFMVK	900
										KMLEGSFMVK	900

HuB1.219_1	910	920	930	940	950
HuB1.219_2	SHHESLISST	QGHKHCCRPO	GPLHRKTRDL	CSLVYLLILP	PEESYDPAKS
HuB1.219_3	GGGWL...				950
HuOBR	901				950
MuOBR	901	KHTASVTOGP	LILLEPETISE	DISVDTSWKN	KDEMAMPTQVV
	901				SEKESITDLEK
					950
					950
HuB1.219_1	960	970	980	990	1000
HuB1.219_2	PSYRNTQE...				1000
HuB1.219_3	951				1000
HuOBR	951				1000
MuOBR	951	GEVICISDQFN	SVNFSEAEGT	EVITYAESQR	QPFVKYATLI
	951				SNSKPSEIGE
					1000
					1000
HuB1.219_1	1010	1020	1030	1040	1050
HuB1.219_2	1001				1050
HuB1.219_3	1001				1050
HuOBR	1001	EQGLINSSVT	KCFSSKNSPL	KDSFSNNSWE	IEAQAFFILS
MuOBR	1001				DQHFPNISPH
					1050
					1050
HuB1.219_1	1060	1070	1080	1090	1100
HuB1.219_2	1051				1100
HuB1.219_3	1051				1100
HuOBR	1051	LTFSEGLDEL	LKLEGNFPEE	NNDXKSIYLY	GVTSIKKRES
MuOBR	1051				GVLLTDKSRV
					1100
					1100
HuB1.219_1	1110	1120	1130	1140	1150
HuB1.219_2	1101				1150
HuB1.219_3	1101				1150
HuOBR	1101	SCPFPAFCLF	TDIRVLQDSC	SHFVENNINL	GTSSKKTFAS
MuOBR	1101				YMPFOFOCST
					1150
					1150
HuB1.219_1	1160	1170	1180	1190	1200
HuB1.219_2	1151				1200
HuB1.219_3	1151				1200
HuOBR	1151	QTHKIMENKH	CDLTV*		
MuOBR	1151				1200
					1200

Figure 3 C



ABSORBANCE

Figure 4

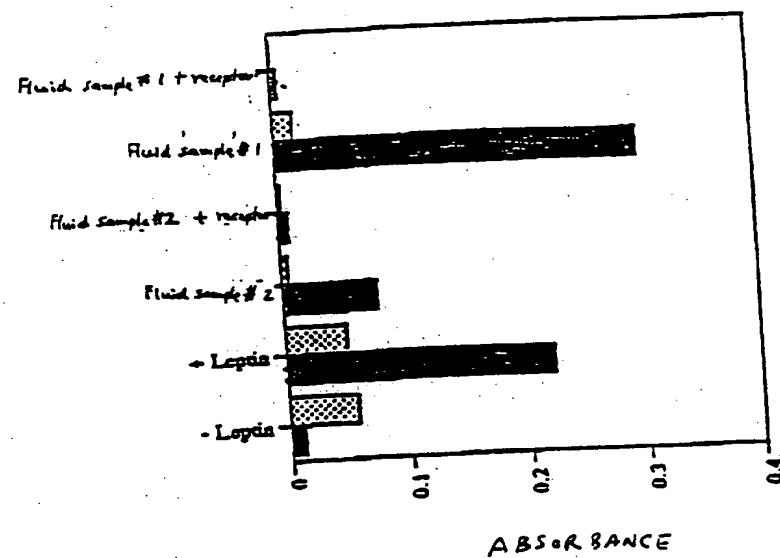


Figure 5

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US97/07676

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12P 19/34; C07H 21/02, 21/04

US CL : 435/6, 91.2; 536/23.1, 24.3, 24.33

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/23.1, 24.3, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TARTAGLIA, L.A. et al., Identification and Expression Cloning of a Leptin Receptor, OB-R. Cell . 29 December 1995. Volume 83, pages 1263-1271, see entire document.	1,9,17, 25,26,30, 32
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A		2-8, 10-16, 18- 24, 27-29, 31, 33

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	
*A*	document defining the general state of the art which is not considered to be of particular relevance
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*O*	document referring to an oral disclosure, use, exhibition or other means
*P*	document published prior to the international filing date but later than the priority date claimed
*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*&*	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 JULY 1997	04.08.97

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DIANNE REES Telephone No. (703) 308-0196
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Form PCT/ISA/210 (second sheet)(July 1992)\*

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/07676

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used).

APS, BIOSIS, BIOTECHABS, BIOTECHDS, CABABPLUS, CANCERLIT, EMBASE, MEDLINE, USPATFULL,

EUROPATFULL, JAPIO, GENBANK, SCISEARCH, LIFESCI, TOXLINE, TOXLIT, DRUGU

search terms: leptin receptor, OB-R, probes, primers, sequence, cloning, diagnosis, mutation detection, alleles, variants, forms, isoforms, ribozymes, antisense, triple helix, screening, drugs